SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Tia1 forms self-perpetuating aggregates in yeast cells

Related to Figure 1

(A) Expression of the *CUP-TIA1-YFP* construct in yeast cells: same levels of the Tia1-Yfp protein are detected in early-, mid- and late-log phases of culture growth.

Transformants of [*pin*$^-$][*psi*$^-$] 74-D694 carrying pCUP-TIA1-YFP were first grown in the plasmid-selective SD-Ura medium for 2 days, and then diluted to OD_{600} 0.1 into SD-Ura supplemented with 50 μM $CuSO_4$, to induce expression of Tia-Yfp (i.e. the cells and growth were exactly as in the experiment described in Figure 1B). Total protein extracts were prepared at 15, 25, 35 hr time points, and separated on the SDS-PAGE gel. Tia-Yfp was detected with anti-Gfp antibodies. Levels of α-tubulin were used for normalization.

(B) Validation of the INI / NNI approach by demonstrating that it allows to reveal the self-propagating nature of the [*PSI*$^+$] prion induced *de novo* by overexpression of the prion domain of the Sup35 protein.

The *de novo* formation of the [*PSI*$^+$] prion can be induced by overexpression of the Sup35 protein or its prion domain, Sup35NM (Chernoff et al., 1993; Derkatch et al., 1996), but such *de novo* formation of [*PSI*$^+$] also requires the presence of another yeast prion, [*PIN*$^+$] (Derkatch et al., 1997; Derkatch et al., 2001; Osherovich and Weissman, 2001). In [*PSI*$^+$] cells, regardless of the presence of [*PIN*$^+$], the presence of the [*PSI*$^+$] prion can be visualized as dot-shaped fluorescent foci following a short-term expression of Sup35 or Sup35NM fused to a fluorescent protein (Patino et al., 1996). These dot-shaped foci represent mature [*PSI*$^+$]. The *de novo* appearance of [*PSI*$^+$] in [*PIN*$^+$][*psi*$^-$] cultures is manifested by the formation of characteristic ring-shaped structures. Cells with rings accumulate gradually after the start of the expression of a

[*PSI*$^+$]-inducing construct, and cells with dot aggregates, indicative of the formation of mature [*PSI*$^+$], appear as the mitotic progeny of cells with ring aggregates (Zhou et al., 2001; Ganusova et al., 2006; Mathur et al., 2010; Tyedmers et al., 2010).

The [*PIN*$^+$][*psi*$^-$] derivative of 74-D694 was transformed with a pCUP-SUP35NM-CFP plasmid, in which expression of *SUP35NM-CFP* was controlled by a copper-inducible promoter. The INI and NNI cultures were grown exactly as in the experiment described in Figure 1D. Percentage of cells with dot- and ring-shaped Sup35NM-Cfp aggregates was determined at the indicated time points. Graphs show averages and standard deviations (AVE +/- SD) based on 5 cultures and ~100 cells counted for each culture for each time point. Confirming the formation of the [*PSI*$^+$] prion during the initial pulse of Sup35NM-Cfp induction, dot-containing [*PSI*$^+$] cells were detected much earlier and remained more abundant in INI cultures, compared to NNI cultures (bottom graph). Conversely, the percentage of cells with rings, which manifest [*PSI*$^+$]s forming *de novo* during the final pulse of Sup35NM-Cfp induction, was not increased in the INI cultures (top graph). Rather, it was reduced compared to NNI cultures, which is expected because the INI cultures contain [*PSI*$^+$] cells that are unavailable for ring formation. (Please note that kinetics of the induction of *de novo* [*PSI*$^+$] formation is not linear and is modulated by the partial toxicity of [*PSI*$^+$]-inducing constructs in [*PSI*$^+$] cells; Derkatch et al., 1996; Zhou et al., 2001; Vishveshwara et al., 2009).

(C) Low-level expression of Tia1-Yfp in the no excess copper medium does not induce new Tia1 foci and does not increase the rate of foci formation during subsequent Tia1-Yfp induction: control for the INI / NNI experiment in Figure 1D.

The cells are the same as in Figures 1A - 1D and S1A: the [*pin*$^-$][*psi*$^-$] 74-D694 derivative transformed with pCUP-TIA1-YFP. The difference between **NI** and **NNI** cultures is in the length of growth in the SD-Ura medium without excess $CuSO_4$ prior to the dilution into the construct-

inducing SD-Ura+50 μM $CuSO_4$ medium. The **NI** cultures were only pre-cultured in SD-Ura for 2 days; i.e. these cultures are equivalent to cultures in Figures 1A, 1B and S1A. The **NNI** cultures were first pre-cultured in SD-Ura for 2 days and then diluted to OD_{600} 0.1 into fresh SD-Ura and grown for 2 days continuously, and for 2 more days with regular dilutions into fresh SD-Ura to maintain logarithmic growth, i.e. these cultures are equivalent to NNI cultures in Figure 1D. After growth in SD-Ura, both NI and NNI cultures were diluted to OD_{600} 0.1 into SD-Ura+50 μM $CuSO_4$, and percentage of aggregate-containing cells was determined at the indicated time points. See Figure S2A for the confirmation of low-level and high-level expression of Tia1-Yfp in SD-Ura without excess $CuSO_4$ and in SD-Ura+50 μM $CuSO_4$, respectively (top panel, compare lanes 2 and 5). Graphs show AVE +/- SD based on 3 cultures and 500 cells counted for each culture for each time point.

(D) Formation of Tia1-Yfp foci is Hsp104-dependent: quantification of the experiment described in Figure 1E.

The *hsp104-Δ* mutant (see Strains in Supplemental Experimental Procedures) and the isogenic control strain [*pin*$^-$][*psi*$^-$] 74-D694 were transformed with pCUP-TIA1-YFP. Cultures were grown exactly as in Figure 1B. Cellular distribution of Tia1-Yfp was monitored from early-log till stationary phase (from ~20 to ~50 hrs of growth in SD-Ura+50 μM $CuSO_4$). At every time point there were at least 20-fold less Tia1-Yfp aggregate-containing cells in the *hsp104-Δ* cultures, and most of these aggregates appeared atypical. Shown is the percentage of cells with Tia-Yfp foci at the last, 50 hr, time point. AVE +/- SD are based on 5 cultures and ~100 cells counted for each culture.

(E) Formation of SDS-resistant oligomers during expression of untagged Tia1 in yeast: oligomerization does not depend on tags attached to Tia1.

The [*pin*$^-$][*psi*$^-$] 74-D694 derivative was transformed with either pCUP-TIA1-YFP or pCUP-TIA1 (see Plasmids in Supplemental Experimental Procedures). Transformants were first grown in the plasmid-selective SD-Ura medium for 2 days, and then diluted to OD_{600} 0.1 into SD-Ura supplemented with 50 μM $CuSO_4$, to induce protein expression (i.e. growth was exactly as in Figures 1A and 1B). Proteins for SDS-AGE analysis were extracted after 40 hours of growth in construct inducing conditions. Numbers indicate the positions of molecular weight markers (see Supplemental Experimental Procedures).

Note: in addition to this experiment, the lack of the effect of tags on Tia1 aggregation is shown by reproducing all major findings with Tia1 constructs carrying genetically unrelated fluorescent protein tags, the Gfp-based (Tia1-Yfp), and the Rfp-based (Tia1-mCherry). For example, fluorescent aggregates with Tia-mCherry ar shown in Figures 1H, S2G; for SDS-AGE analysis compare the left panel of Figure 3C and Figure S3B; for co-immunoprecipitation experiments - see Figures 6C and S6C.

(F) Dcp2 foci, indicative of the presence of Dcp2-containg RNP particles, are detected in most cells in the *hsp104-Δ* strain where formation of Tia1 foci is almost completely abolished.

The gene encoding the Hsp104 chaperone was disrupted in the *DCP2-YFP* [*pin*$^-$][*psi*$^-$] 74-D694 strain where the *DCP2-YFP* was substituted for the *DCP2* ORF (see Strains in Supplemental Experimental Procedures). The resulting *hsp104-Δ DCP2-YFP* [*pin*$^-$][*psi*$^-$] 74-D694 mutant and the control strain (*DCP2-YFP* [*pin*$^-$][*psi*$^-$] 74-D694; same as in Figure 1H) were transformed with the pCUP-TIA1-mCherry plasmid. Cultures were grown exactly as in Figure 1H. Small punctate Dcp2-Yfp foci could be detected in most cells, whereas Tia1-mCherry was evenly distributed in

the cytoplasm, consistent with data shown in Figures 1E and S1D. Images of a representative group of cells were taken 50 hrs after Tia1-mCherry expression was induced.

(G) Tia1 dot aggregates partially co-localize with the Pub1 protein.

The pCUP-TIA1-mCHERRY plasmid was introduced into a strain where the *PUB1-GFP* was substituted for the *PUB1* ORF (see Strains in Supplemental Experimental Procedures). Culture growth conditions were exactly the same as in Figure 1H, where co-localization with Dcp2 is shown. Images of two representative cell groups were taken 30 hours after inducing the expression of Tia1-mCherry. Co-localization with Pub1 was observed in most cells with bright Tia1 dots. However, aggregation of Tia1 did not induce a shift of all Pub1 to Tia1 dots, most of Pub1 remained distributed throughout the cell with only a fraction of the protein concentrating in Tia1 foci.

Figure S2.

Related to Figure 2

(A) Expression of *CUP-TIA1-YFP* and *CUP-SUP35NM-CFP* in media with and without 50 μM $CuSO_4$.

Related to Figure 2A. Expression of Sup35NM-Cfp and Tia1-Yfp is the same in cultures expressing each protein separately or co-expressing both proteins (compare lanes 4 and 6 for Sup35NM-Cfp, and lanes 5 and 6 for Tia1-Yfp).

Related to Figures 1D and 2D. Amounts of the Tia1-Yfp and Sup35NM-CFP proteins return to the basal level after yeast are transferred from media supplemented with 50 μM $CuSO_4$ to media without excess $CuSO_4$ (compare lanes 1 – 3 and 7 - 9).

The [*pin*$^-$][*psi*$^-$] 74-D694 strain was co-transformed with pCUP-TIA1-YFP and pCUP-CFP (T), pCUP-SUPNM-CFP and pCUP-YFP (S), or pCUP-TIA1-YFP and pCUP-SUPNM-CFP (S/T). Yeast were first grown in plasmid selective SD-Ura,Leu medium without excess copper (-$CuSO_4$; lanes 1-3), then diluted to OD_{600} 0.1 into SD-Ura,Leu supplemented with 50 μM $CuSO_4$ (+ $CuSO_4$; lanes 4-6), and then diluted again into SD-Ura,Leu without excess copper (-$CuSO_4$; lanes 7-9). Growth in each medium was for 2 days. Total protein extracts were prepared from each culture in mid-log phase and separated by SDS-PAGE. Tia-Yfp was detected with anti-Tia1 antibodies; Sup35NM-Cfp was detected with anti-Sup35 antibodies recognizing Sup35N (see Supplemental Experimental Procedures for Sup35 antibodies; full-length chromosomally encoded Sup35 also recognized by this antibody is larger and not included in the gel fragment shown in the figure). Levels of α-tubulin were used for normalization.

The possibility that in a small subset of cells high-level expression of Tia1-Yfp or Sup35NM-Cfp is not shut down upon switching to the medium without excess $CuSO_4$ was tested by the fluorescent microscopy analysis of ~10,000 cells for each culture: no such cells were detected (data not shown).

(B) Growth curves for cultures expressing Tia1-Yfp and Sup35NM-Cfp: expression of Tia1-Yfp causes only slight growth inhibition.

Transformants of [*pin*$^-$][*psi*$^-$] 74-D694 carrying pCUP-TIA1-YFP, pCUP-SUP35NM-CFP, or control pCUP-YFP and pCUP-CFP plasmids were grown in the plasmid-selective SD-Ura,Leu medium for 2 days and then diluted to OD_{600} 0.1 into SD-Ura, Leu supplemented with 50 μM $CuSO_4$, to induce the expression of the *CUP1*-driven constructs. Graphs show AVE +/- SD based on 3 cultures for each plasmid combination.

(C) Expression of Tia1-Yfp does not cure [*PSI*$^+$].

A weak [*PSI*$^+$][*pin*$^-$] 74-D694 strain was transformed with pCUP-TIA1-YFP or the control pCUP-YFP plasmid. Transformants were grown in the plasmid-selective SD-Ura medium for 2 days and then diluted to OD_{600} 0.1 into SD-Ura supplemented with 50 μM $CuSO_4$, to induce the expression of the *CUP1*-driven constructs. After ~30 generations of growth in SD-Ura+50 μM $CuSO_4$, which involved 3 more transfers to fresh medium to maintain growth, yeast were plated onto YPD medium where weak [*PSI*$^+$] colonies are pink and [*psi*$^-$] colonies that lost the prion are red. Images were taken after 3 days of growth on YPD at 30^0C. Graph shows fraction of red [*psi*$^-$] colonies; AVE +/- SD are based on 4 cultures for each plasmid with ~1000 colonies counted for each culture.

Similar data for strong [*PSI*$^+$] are not shown.

(D) Preventing the entry into late-log delays the formation of Tia1-Yfp / Sup35NM-Cfp lines.

The cells were the same as in Figures 2B, 2C and 2D: co-transformants of [*pin*$^-$][*psi*$^-$] 74-D694 strain with pCUP-TIA1-YFP and pCUP-SUP35NM-CFP. Experiment was performed exactly as described in Figure 1C: cells were first pre-cultured in SD-Ura,Leu for 2 days; then cultures were either grown continuously in SD-Ura,Leu+50 μM $CuSO_4$, or diluted to OD_{600} 0.1 into SD-Ura,Leu+50 μM $CuSO_4$ every 10-13 hrs. Percentage of aggregate-containing cells was determined after ~ 34 hours of growth in SD-Ura,Leu+50 μM $CuSO_4$. Graphs show AVE +/- SD based on 5 cultures with 1000 cells counted for each culture.

(E) Formation of Tia1-Yfp / Sup35NM-Cfp line aggregates is Hsp104-dependent: images of a representative group of cells for experiment described in Figure 2E.

The *hsp104-Δ* mutant (see Strains in Supplemental Experimental Procedures) and the isogenic control strain [*pin*$^-$][*psi*$^-$] 74-D694 were co-transformed with pCUP-TIA1-YFP and pCUP-SUP35NM-CFP. Cultures were grown exactly as in Figure 2B. Cellular distribution of Tia1-Yfp and Sup35NM-Cfp was monitored from early-log till stationary phase (from 20 to 50 hrs of growth since the start of the expression of the constructs). At every time point, in the *hsp104-Δ* cultures there were no cells with straight lines, and cells with atypical aggregates were at least 50-fold less frequent than in control cultures. Shown are images of the *hsp104-Δ* cells taken after ~50 hrs of co-expression of Tia1-Yfp and Sup35NM-Cfp (same time point as in Figure 2E).

(F) Formation of line structures when Tia1-Yfp is expressed in the cells overexpressing untagged Sup35.

Transformants of the [*pin*$^-$][*psi*$^-$] 74-D694 strain with pCUP-TIA1-YFP and pCUP-SUP35 were grown as in Figures 1A and 2B. An image of a representative cell group was taken in the late-log phase.

(G) A switch of fluorophore does not affect the ability of Tia1 to form lines when co-expressed with Sup35.

Transformants of the [*pin*$^-$][*psi*$^-$] 74-D694 strain with pCUP-TIA1-mCherry and pCUP-SUP35NM-Cfp were grown as in Figures 1A and 2B. An image of a representative cell group was taken in the late-log phase. Only mCherry and DIC images are shown.

Figure S3. Tia1 alone, Sup35 alone and Tia1 / Sup35 form three distinct self-propagating structures with SDS-resistant oligomers of different size

Related to Figure 3

(A) Formation of Tia1 / Sup35 line aggregates is not affected by switching from glucose to galactose: control experiment for Figure 3A.

In the experiment described in Figure 3A, formation of Tia1-Yfp dot foci and Tia1-Yfp / Sup35NM-Cfp line aggregates is analyzed in cultures that were transferred from SD-Ura,Leu+50 μM $CuSO_4$ medium containing 2% glucose as the only carbon source to SGalSR-Ura,Leu+50 μM $CuSO_4$ medium containing three carbon sources: 2% galactose (to induce constructs controlled by the *GAL* promoter), and 1% sucrose and 1% raffinose (to facilitate growth without inhibiting *GAL* promoter, because the 74-D694 strain grows poorly in liquid media where galactose is the only carbon source). This is the only experiment in the study where galactose-inducible constructs are used - in all other experiments Tia1-Yfp and Sup35NM-Cfp and derivative constructs are controlled by the *CUP1* promoter and yeast are grown in glucose medium. To validate the experimental approach used in Figure 3A, this control tests whether Tia1 and Tia1 / Sup35 self-propagating structures form at the same rate during growth in glucose medium, and upon switching from glucose to galactose / sucrose / raffinose medium. Parallel cultures of [*pin*$^-$][*psi*$^-$] 74-D694 transformed with pCUP-TIA1-YFP and pCUP-SUP35NM-CFP were pre-cultured in SD-Ura,Leu for 2 days, and then diluted to OD_{600} 0.1 into SD-Ura,Leu+50 μM $CuSO_4$ and grown for 20 hrs (same length of incubation as in Figure 3A). Then cultures were transferred either to SGalSR-Ura,Leu+50 μM $CuSO_4$, (Glu to Gal; exactly the same experimental conditions as in Figure 3A), or to SD-Ura,Leu+50 μM $CuSO_4$ (Glu only control), and growth was continued for a total of 50 hrs. Percentage of cells with line aggregates was determined at the indicated time points. Shown are AVE +/- SD based on 3 cultures for each growth condition. As in Figure 3A, growth rates were very similar for all cultures (data not shown).

(B) Analysis of SDS-resistant oligomers formed by Tia1 and Sup35 in the derivatives of the 74-D694 strain with different combinations of [*PSI*$^+$] and [*PIN*$^+$] prions.

Shown is the complete image of the gel, from which the middle and right panels of Figure 3C were derived, so the experiment is described in Figure 3C legend. In brief, proteins were extracted from late-log cultures grown in the construct-inducing medium, and total protein extracts were separated by SDS-AGE for subsequent Western blot analysis. The presence of [*PSI*$^+$] and [*PIN*$^+$] prions in the 74-D694 derivatives is indicated above the gel. Expressed proteins are labeled as "+". Samples that were boiled before loading on the gel are indicated by and asterisk (*). Anti-Gfp antibody was used for the detection of Sup35NM-Cfp and Tia1-Yfp. Arrow, arrowhead and dashed arrow indicate, respectively, oligomers corresponding to Tia1/Sup35 lines, Tia1 dots and [*PSI*$^+$]. Numbers indicate the positions of molecular weight markers (see Supplemental Experimental Procedures).

(C) Specificity of co-immunoprecipitation of Tia1-mCherry with Sup35NM-Cfp: control for experiments described in Figures 3D and 6C.

The goal of this experiment is to confirm that Tia1-mCherry co-immunoprecipitates with Sup35NM-Cfp but not with Cfp.

Same protein extracts were used for the experiments shown here and in Figures 3D and 6C.

Cultures of transformants of [*pin*$^-$][*psi*$^-$] 74-D694 with pCUP-TIA1-mCHERRY and either pCUP-CFP or pCUP-SUP35NM-CFP were pre-cultured in SD-Ura,Leu for 2 days and then diluted to OD_{600} 0.1 into SD-Ura,Leu+50 μM $CuSO_4$. Proteins were extracted ~36 hrs after the induction of protein expression. Then they were immunoprecipitated with anti-Gfp, (shown here and in Figure 3D), or with anti-Tub1 (Figure 6C). Anti-Gfp immunoprecipitates were separated by SDS-PAGE (shown here) or SDS-AGE (Figure 3D). Anti-Tub1 immunoprecipitates were separated by

SDS-PAGE (Figure 6C). The Input panel shows α-tubulin levels in protein extracts (shown here is a fragment of the Input panel from Figure 6C). Sup35NM-Cfp (arrow), Tia1-mCherry (arrowhead) and Cfp (dashed arrow) were detected, respectively with anti-Gfp, anti-Rfp and anti-Gfp. Empty bead control for Tia1-mCherry is included in the bottom panel; for other empty bead controls see Figures S6E and S6G. Each variant of experiment was repeated at least 2-3 times. The empty bead control for anti-Gfp in the rightmost panel of Fig. S6G is applicable to both IP::Gfp and IP::Tub1.

(D) Analysis of Dcp2 aggregation in cultures containing Tia1-mCherry dot foci: Tia1 dot foci are not co-aggregates of Tia1 and Dcp2.

In cells co-expressing Tia1-mCherry and Sup35NM-Cfp, fluorescent microscopy reveals co-localization of these proteins in line structures (Figure 2B), whereas SDS-AGE analysis reveals that in line-containing cultures Tia1-mCherry and Sup35-NM-Cfp form SDS-resistant oligomers (Figure 3C) that co-aggregate (Figure 3D). In cultures where Tia1-mCherry is expressed alone, Dcp2-Yfp co-localizes with the majority of dot-shaped foci formed by Tia1-mCherry (Figure 1H). However, Dcp2-Yfp is detected in dot-shaped granules even when the formation of Tia1-mCherry foci is blocked by the disruption of *HSP104* (Figure S1F). In this experiment, we use SDS-AGE analysis to test whether Dcp2 forms SDS-resistant oligomers in cultures containing Tia1 dot foci.

The pCUP-TIA1-mCherry plasmid was introduced into a strain where the *DCP2-YFP* was substituted for the *DCP2* ORF (*DCP2-YFP* [*pin*$^-$][*psi*$^-$] 74-D694; see Strains in Supplemental Experimental Procedures). Same strain was used in Figure 1H, in which co-localization of Dcp2 with Tia1 foci was detected. Cultures were grown as in experiments described in Figure 1H. Proteins were extracted 40 hrs after the expression of Tia1-mCherry was induced. Protein extracts were incubated in 2% SDS either at room temperature (RT) or at 100^{0}C (Boil), and

separated by SDS-AGE (same samples were used for the lanes in the right and left panels). Anti-Rfp and anti-Gfp were used for the detection of Tia1-mCherry and Dcp2-Yfp, respectively. As expected, Tia1-mCherry was mostly in SDS-resistant heat-liable oligomers. However, only monomeric protein was detected for Dcp2-Yfp. This result indicates that Dcp2 does not form SDS-resistant oligomers when Tia1 forms dots, that Tia1 and Dcp2 co-localization is not due to prion-like co-aggregation, and that self-perpetuating Tia1 structures are not equivalent to Dcp2-containing RNP granules. Experiment was repeated 4 times. Loading controls are not shown, but note expression of both Dcp2-Yfp and Tia1-Rfp in Figure 1H. Numbers indicate the positions of molecular weight markers (see Supplemental Experimental Procedures).

Figure S4. Evolutionarily conservation and specificity of Tia1 / Sup35 interaction

Related to Figure 4

(A) Co-expression of Sup35NM-Cfp and Pub1-Yfp leads to the appearance of line aggregates: co-localization data for the right panel of Figure 4A.

Experiment was performed as in Figure 2B, except that [*pin*$^-$][*psi*$^-$] 74-D694 was transformed with pCUP-PUB1-YFP and pCUP-SUP35NM-CFP. Images were taken in late-log. Arrows point at cells with Pub1 / Sup35 line aggregates.

(B) Formation of SDS-resistant aggregates in yeast cells expressing Pub1-Yfp.

To the best of our knowledge, formation of SDS-resistant aggregates by full-length Pub1 was not reported before, but there is previously published evidence on prion-like aggregation of Pub1 or its prion domain. Alberti et al. (2009) observed SDS-resistant aggregates formed by the presumptive prion domain of Pub1 in a [*PIN*$^+$] strain with a different genetic background. Urakov et al. (2010) observed sarcosyl-resistant Pub1 aggregates in the same genetic background but only in the presence of the [*PSI*$^+$] or [*PIN*$^+$] prions.

See (C) below for the description of the experiment.

(C) Formation of SDS-resistant oligomers in yeast cells co-expressing Pub1-mCherry and Sup35NM-Cfp.

In (B) and (C): The [*pin*$^-$][*psi*$^-$] 74-D694 strain was transformed with either only pCUP-PUB1-YFP (B), or co-transformed with pCUP-PUB1-mCHERRY and pCUP-SUP35NM-CFP (C). Cultures were grown in construct-inducing conditions until late-log (same growth conditions as in Figures 1A, 2B and 4A). SDS-AGE analysis was performed as in Figures 3C and 4B. In (S4B) membrane was hybridized with anti-Gfp. In (S4C) membrane was hybridized with anti-Gfp (left) and anti-Rfp antibodies (right); same membrane was used for both hybridizations. RT and Boil indicate, respectively, incubation at room temperature and 100^0C in 2% SDS buffer.

(D) Interaction between Tia1 and mammalian homolog of Sup35, Gspt2: co-localization data for Figure 4D.

Experiment was performed as in Fig. 2B, except that [*pin*$^-$][*psi*$^-$] 74-D694 was transformed with pCUP-TIA1-YFP and pCUP-GSPT2-CFP. In pCUP-GSPT2-CFP full-length *GSPT2* is fused to *CFP.*

Upon co-expression, Gspt2-Cfp and Tia1-Yfp form both dot and line structures. By late-log / early stationary phase >95% cells contain dot or line aggregates, or both. Lines that are well seen for Gspt2-Cfp can be straight (left panel) or wiggly, sometimes with several lines coming out of the same point (middle panel). Tia1-Yfp forms very thin lines co-localizing with Gspt2-Cfp lines. Also, Tia1 dot aggregates tend to cluster along the lines. In some cells this clustering is profound, so that Tia1-Yfp dots have a tight bead-on-the-string pattern (e.g. upper cell in the middle panel). Gspt2-Cfp dot aggregates can be either punctate or clumpy and usually co-localize with Tia1-Yfp dots (right panel).

(E) Tia1-Yfp does not form line structures when co-expressed with the first exon of the huntingtin protein encompassing polyQ stretches of different length.

The [*pin*$^-$][*psi*$^-$] 74-D694 strain was transformed with pCUP-TIA1-YFP and a pGPD-QXX-SUP35M-GFP construct where XX in the length of the polyQ stretch (kindly provided by S. Lindquist, MIT; Krobitsch and Lindquist, 2000; see Supplemental Experimental Procedures for the description of the constructs). Cultures were grown as in Figures 1A and 2B, and images of representative cell groups were taken in late-log phase. Shown are the Yfp channel images for the co-transformants bearing the Q25, Q47 and Q103 variants. In these images Gfp fluorescence from polyQ constructs bleeds into the Yfp channel, so aggregates in the images are both Tia1-Yfp and polyQ-Gfp; just note the lack of line structures.

Figure S5.

Related to Figure 5

Schematic representation of the Sup35 protein is the same as in Figure 5B; see Results and Figure 5B legend for the description.

(A) Importance of K/E patches located in the M domain of Sup35 between aa 167-220 for the formation of line aggregates: deletion of K/E sequences blocks the formation of lines during co-expression with Tia1.

The pCUP-TIA1-YFP and one of the following constructs, pCUP-SUP35NM-CFP, pCUP-SUP35NM(N+166-200)-CFP or pCUP-SUP35NM(N+166-200-ΔK/E)-CFP, were introduced into the [*psi*$^-$][*pin*$^-$] 74-D694 strain. The Sup35NM(N+166-200)-Cfp and Sup35NM-Cfp constructs are the same as in Figure 5B. For the new Sup35NM(N+166-200-ΔK/E)-Cfp construct, we deleted

all three mixed K/E stretches underlined in Table S1 (see below) as well as two KE dipeptides located between the first and the second K/E stretches. The amino acid sequence of the M region in this construct is the following: ESDSAETPTPTKVEEPVPVQTSELPKVEDLKI. Growth conditions were exactly the same as in Figure 5B where analysis of other Sup35 deletion constructs is presented. Images of representative cells groups were taken ~30 hours the expression of the constructs was induced. While line formation was completely inhibited in cells co-expressing Tia1-Yfp and Sup35NM(ΔKE)-Cfp, formation of Tia1-Yfp dot aggregates was not affected.

(B) Formation of line aggregates by Tia1 and the prion domain of the Sup35 protein does not depend upon the presence of full-length Sup35: same experiment as in Figure 5B but performed in the *sup35-ΔNM* strain

Experiments were performed in the [*pin*$^-$][*psi*$^-$] *sup35-ΔNM* mutant of the 74-D694 strain transformed with pCUP-TIA1-YFP and one of the following constructs: pCUP-SUP35NM-CFP, pCUP-SUP35(N+166-254), or pCUP-SUP35(N+166-220). Cultures expressing Tia1-YFP and the indicated Sup35-Cfp constructs were grown as in Figures 1A, 2B and 5B. Images were taken in late-log. The top images are the same as in Figure 5B. See Results and Table S1 for the detailed description of regions within the prion domain of Sup35 chosen for these constructs.

Figure S6. Self-propagating aggregates of Sup35 and Tia1/Pub1 are associated with the tubulin cytoskeleton

Related to Figure 6

(A) Growth curve for cultures grown in the presence of 20 μg/ml of microtubule-disrupting drug benomyl: disruption of the formation of line aggregates is observed at a concentration when benomyl causes mild growth inhibition.

Transformants of [*pin*$^-$][*psi*$^-$] 74-D694 carrying pCUP-TIA1-YFP and pCUP-SUP35NM-CFP were pre-cultured in the plasmid-selective SD-Ura,Leu medium for 2 days and then diluted to OD_{600} 0.1 into SD-Ura,Leu+50 μM $CuSO_4$ supplemented with either 20 μg/ml benomyl diluted in DMSO (Benomyl), or just DMSO (DMSO control). Graphs show AVE +/- SD for optical density, OD_{600}, based on 3 cultures.

(B) Formation of Tia1 / Sup35 line aggregates is not affected by actin-depolymerizing drug latrunculin A.

Several previous studies implicated actin cytoskeleton in the formation and maintenance of the [*PSI*$^+$] prion (Bailleul-Winslett et al., 2000; Ganusova et al., 2006; Mathur et al., 2010), so we asked if disruption of the actin cytoskeleton also affects the formation of Tia1 / Sup35 lines. The obtained negative result is consistent with our finding that Tia1 / Sup35 line structures and [*PSI*$^+$] are independent (see Fig. 3B). This result also underscores the specificity of Tia1/Sup35 interaction with α-tubulin.

Transformants of [*pin*$^-$][*psi*$^-$] 74-D694 carrying pCUP-TIA1-YFP and pCUP-SUP35NM-CFP were grown in plasmid-selective SD-Ura,Leu medium for two days, then yeast were diluted to OD_{600} 0.1 into SD-Ura,Leu+50 μM $CuSO_4$ and grown to OD_{600} 0.5 (~ 10 hours; i.e. before SDS-resistant oligomers and visible aggregates appeared). The 10 mM stock of Latrunculin A in DMSO was added to these early-log cultures to the final concentration of 40 μM (+ Latrunculin A). Equal amount of DMSO was added to the control cultures (- Latrunculin A). Aggregate formation was scored multiple times until the stationary phase; representative images taken 20

hours after the addition of the drug are shown. Cultures had to be grown to early-log before the addition of Latrunculin A due to significant growth inhibition caused by the drug; similar approach was previously used to detect the effect of Latrunculin A on [*PSI*$^+$] in the same genetic background (Bailleul-Winslett et al., 2000).

(C) Co-immunoprecipitation of Sup35NM-Cfp and Tia1-Yfp with anti-Tub1 antibody: same experiment as in Figure 6C but proteins carry different fluorescent tags.
Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 transformants expressing the indicated proteins were grown as in Figures 1A, 2B and 6C. Top panel shows amounts of Tub1 in protein extracts used for immunoprecipitation. Material from the same co-immunoprecipitation experiment was used for Western blot analyses in all tree bottom panels. Arrow indicates the position of Sup35NM-Cfp and Tia1-Yfp detected with anti-Gfp (the size of the proteins is similar). Arrowhead indicates the position Tia1-Yfp detected with the Tia1-specific antibody. Dashed arrow indicates the position of full-length Sup35 encoded by the chromosomal *SUP35* gene (detected with the BE4 antibody recognizing Sup35C); in this panel every odd lane is the immunoprecipitate with the anti-Tub1 antibody, and every even lane is the “empty bead” control.

(D) Neither Sup35, nor Pub1 co-immunoprecipitate with actin cytoskeleton components in the anti-Act1 pull-down: specificity control for the association of Sup35 and Pub1 with tubulin.
Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 (left panel) or of the strain where the *PUB1-GFP* was substituted for the *PUB1* ORF (right panel) were grown in YPD till late-log (i.e. proteins were expressed at physiological level, and growth conditions were the same as in Figure 6D showing the association of Sup35 and Pub1-Yfp with tubulin). IN, IP and C indicate, respectively inputs, anti-Act1 immunoprecipitates and “empty bead” controls. Numbers indicate positions of molecular

weight markers. Same membranes were used for Western blot analyses in top and bottom panels. Antibodies used for protein detection are indicated on the figures next to the gels.

(E) Yfp and Cfp proteins do not co-immunoprecipitate with anti-Tub1.

This figure also serves as an "empty bead" control for Yfp and Cfp.

Protein extracts were prepared form a [*psi*$^-$][*pin*$^-$] 74-D694 culture co-expressing Yfp and Cfp proteins grown as in Fig. 2B. The extract is the same as in Figures 6C, S3C and 3D. Proteins were immunoprecipitated with anti-Tub1 or with "empty beads" lacking the antibody. IN – input; IP- immunoprecipitates; C – "empty bead" control. Number indicates the molecular weight marker. Arrowhead points at the Cfp / Yfp position.

(F) Sup35 co-distributes with tubulin on sucrose gradient

Cell lysate was prepared from a culture of [*pin*$^-$][*psi*$^-$] 74D-694 grown in YPD till late-log (i.e. all proteins are expressed at physiological levels). Sucrose gradient analysis was performed as described in Supplemental Experimental Procedures. Antibodies used for protein detection are listed next to the panels. IN indicates input (20 µg of lysate). Numbers indicate positions of molecular weight markers. Both Sup35 and tubulin were detected in fractions 1 trough 5 with the highest concentration in fraction 3, consistent with their involvement in macromolecular complexes, and with their presence in the same complex. Rnq1 was used as a control: while capable of taking on an aggregated prion conformation, [*PIN*$^+$], in [*pin*$^-$] strains Rnq1 is monomeric and stays mostly on the top of sucrose gradients (Kadnar et al., 2010).

(G) "Empty bead" controls for Figure 6.

Two left panels and the rightmost panel: protein extracts are from a [*psi*$^-$][*pin*$^-$] culture co-expressing Tia1-mCherry and Sup35NM-Cfp; the extract is the same as in Figures 6C, S3C and

3D. Third panel from the left: protein extract is from the *sup35-ΔNM* strain where full-length Sup35 is expressed from a centromeric plasmid, in which *SUP35* is controlled by its original promoter; the extract is the same as in Figure 6G. For all panels: IN - input; IP - immunoprecipitates; C – "empty bead" control with beads lacking the antibody.

All IPs included in this figure are for anti-Tub1 beads, but the "empty bead" controls are also valid for immunorecipitations with anti-Gfp beads.

The anti-Sup35C antibody used in Figure 6G is BE4, the same anti-Sup35 antibody is used in Figures 6D, 6E and bottom panel of Figure S6C, where the "empty bead" controls are shown.

For "empty bead" controls for Yfp and Cfp see (E) above.

Figure S7. Tubulin-associated complex recruited by Pub1 and Sup35 encompasses *TUB1* mRNA and components of translational machinery indicative of the presence of the ribosomes

Related to Figure 7

(A) Presence of Sup35 in the Tub1-associated complex depends upon Pub1.

The key difference between this experiment and the experiment shown in Figure 7A is that here we compare two isogenic strains: the [*pin*$^-$][*psi*$^-$] 74-D694 strain, which expresses Pub1 at physiological level from the chromosomal *PUB1* gene (WT), and the *pub1-Δ* mutant obtained in this strain (see Strains in Supplemental Experimental Procedures). In Figure 7A the same *pub1-Δ* mutant is used, but for the control, instead of using the parental wild type strain, the *PUB1* ORF is re-introduced on a plasmid under the control of the *CUP1* promoter.

Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 and of the isogenic *pub1-Δ* mutant were grown in YPD to late-log.

Top: left panel shows amounts of Sup35 in cell lysates used for immunoprecipitation; right panel

shows amount of Sup35 pulled down by the anti-Tub1 antibody; arrow points at the band corresponding to the full-length endogenous Sup35. Bottom: graph shows AVE +/- SD deviations based on 4 experiments normalized to Sup35 immunoprecipitation by anti-Tub1 in wild type 74-D694. See Figure 6D (left) for the "empty bead" control.

(B) Association of Sup35 with α-tubulin in the *pub1-Δ* strain is restored by the re-introduction of either *PUB1* or its mammalian homolog, *TIA1*.

For co-immunoprecipitation with anti-Tub1, the *pub1-Δ* mutant of [*pin*$^-$][*psi*$^-$] 74-D694 was transformed with either the *YFP*-expressing vector, or plasmids encoding the ORFs for *PUB1* or *TIA1* fused to *YFP*. All constructs are driven by the *CUP1* promoter, and cultures were grown as in Fig. 1A. Arrows point at bands corresponding to Yfp, Pub1-Yfp and Tia1-Yfp in lanes 1, 2 and 3, respectively. Arrowhead points at the band corresponding to the Sup35 protein detected with anti-Sup35 (BE4). See Figures S6G and 6D (left) for the "empty bead" control.

(C) Co-immunoprecipitation of the initiation factor eIF4E with α-tubulin: "empty bead" control for the experiment in Figure 7C.

For co-immunoprecipitation with anti-Tub1, a culture of [*pin*$^-$][*psi*$^-$] 74-D694 was grown in YPD till late-log. IN, IP and C indicate, respectively, cell lysate input, immunoprecipitate and "empty bead" control. Arrowhead points at the band corresponding to the eIF4E protein, which was detected with anti-eIF4E.

(D) Co-immunoprecipitation of the Pab1 with α-tubulin.

Pab1 is the poly(A) binding protein, a component of the 3'-end RNA-processing complex where it controls the length of poly(A) tail. Pab1 also interacts with the eIF4G initiation factor thus

mediating interactions between the 5'-cap structures and 3'-tail during translation. For co-immunoprecipitation with anti-Tub1, a culture of [*pin*$^-$][*psi*$^-$] 74-D694 was grown in YPD till late-log. IN, IP and C indicate, respectively, cell lysate input, immunoprecipitate and "empty bead" control. Pab1 was detected with the anti-Pab1 antibody.

(E) Co-immunoprecipitation of the 26S ribosomal RNA with α-tubulin: controls for experiments in Figure 7D.

Top panel: complete gel for lanes 3 – 6 of Figure 7D. C indicates the "empty bead" control with 26S primers. Bottom panel: complete gel for lane 7 of Figure 7D. –RT is the control without reverse transcriptase and with 26S primers.

Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 and the isogenic *pub1-Δ* mutant were grown in YPD till late-log. Anti-Tub1 immunoprecipitates or "empty bead" controls (C) were used for the RT-PCR with the primers specific for the 26S rRNA (26S) or for the large ribosomal subunit RNA precursor (ITS). In the former case the primers would amplify the same 125 bp fragment corresponding to the D1 region of the 26S rRNA from both the mature rRNA and rRNA precursors (or rDNA). In the latter case the forward primer is homologous to the internal transcribed sequence 1 (ITS1), which is present in the precursor but is absent in the mature rRNA, and the reverse primer is complimentary to one of the 26S primers, so the 840 bp fragment would only be amplified from the rRNA precursor (or rDNA), but not from mature rRNA. Amplification of 26S but not the ITS fragment from the Tub1 immunoprecipitates confirms the presence of only mature 26 rRNA in the complex. See Supplemental Experimental Procedures Primers Table for primer sequences.

(F) Co-immunoprecipitation of the *TUB1* mRNA with α-tubulin is modulated by Pub1: controls for experiments shown in Figure 7E.

Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 (WT) and the isogenic *pub1-Δ* mutant were grown in YPD till late-log. RT-PCRs with the *TUB1* primers # 398 and 399 were performed in anti-Tub1 immunoprecipitates (IP::Tub1), or in the "empty bead" control (C). –RT indicates a control without reverse transcriptase but with the *TUB1* primers.

(G) Abundant mRNAs for histone proteins H4 (*HHF2*) and H2A (*HTA1*), and for elongation factor eEF-1α (*TEF1*) and the large subunit ribosomal protein Rpl28 (*RPL28*) are not present in the tubulin-associated Sup35 / Pub1 complex: negative control for Figure 7E.

To see if association of the *TUB1* mRNA with the tubulin-associated Sup35 / Pub1 complex is specific, two sets of control mRNAs were chosen. (i) The *HHF2* and *HTA1* mRNAs encoding nuclear histone proteins H4 and H2A, respectively (two top panels in this figure). Neither of these mRNAs is expected to be present in the complex involved in localized protein synthesis of cytoskeletal components. (ii) The mRNAs that encode components of the translational machinery, including the extremely abundant elongation factor EF-1α (*TEF1*) and large subunit ribosomal protein Rpl28 (*RPL28*) (two bottom panels in this figure), and the major components of the translation termination factor (*SUP35* and *SUP45*) (Figure 7E and data not shown). Among the components of the translational machinery, Sup35 and Rpl28 proteins are present in the complex, while Sup45 and EF-1α are expected to be present. Data on the relative abundance of yeast mRNAs is from Holstege et al. (1998). The fact that none of these mRNAs was detected by RT-PCR in anti-Tub1 immunoprecipitates indicates that a highly specific subset of mRNAs, including *TUB1*, is accumulated in the complex.

Cultures of [*pin*$^-$][*psi*$^-$] 74-D694 were grown in YPD till late-log and lysates were immunoprecipitated with anti-Tub1 exactly as in Figure 7E. Primers used for RT-PCR are listed in the Primer Table in Supplemental Experimental Procedures. IN indicates RT-PCR of the

input; +RT and C indicate, respectively, RT-PCR of the immunoprecipitate and of the “empty bead” control. –RT indicates the control reaction for the immunoprecipitate without reverse transcriptase but with primers. Numbers indicate positions of molecular weight markers.

(H). The components of the Tub1 / Sup35 / Pub1 / translational machinery / *TUB1* mRNA complex are not held together by RNA-based interactions: RNAse A treatment does not dissolve the non-ribosomal components of the complex.

Cell lysate was prepared from [*pin*$^-$][*psi*$^-$] 74-D694 (the top panel and two bottom panels) or from an isogenic strain where *PUB1* ORF was substituted for *PUB1-YFP* (second panel). Cultures were grown in YPD till late-log. Experimental samples (marked by +) were incubated for 1 hr at room temperature with 0.1 mg/ml RNAse A prior to anti-Tub1 immunoprecipitation. For the control samples the incubation was as in all other co-IP experiments, without RNAse A (marked by -). Antibodies used for immunodetection are indicated next to the panels.

Supplemental Table S1. Amino acid sequences of N-terminal extensions of the *S. cerevisiae* Sup35 protein and its mouse homologs, Gspt1 and Gspt2*

YEAST	Sup35N**	1 MSDSNQGNNQQNYQQYSQNGNQQQGNNRYQGYQAYNAQAQ 40
		41 PAGGYYQNYQGYSGY QQGGYQQYN PDAGYQQQYN PQGGYQQYN PQGGYQQQFN PQGGRG 99
		100 NYKNFNYNNNL QGYQAGFQPQSQG 123
	Sup35M	124 MSLNDFQKQQKQAAPKPKKTLKLVSSSGIKLANATKKVGTKPA 166
		167 ESDKKEEEKSAETKEPTKEPTKVEEPVKKEEKPVQTEEKTEEKSELPKVEDLKISE 222
		223 STHNTNNANVTSADALIKEQEEEVDDEVVND 253
MOUSE	Gspt1 part corresponding to Sup35NM***	1 MDPSSGGGGGGGGGSSSSSDSAPDCWDQTDMEAPGPGPCGGGGSGSGSMAAVAEAQRENLSAA FSRQLNVNAKPFVPNVHAAEFVPSFLRGPAQPPLSPAGAAGGDHGAGSGAGGPSEPVESSQDQSCEG SNSTVSMELSEPVVENGETEMSPEESWEHKEEISEAEPGGGSSGDGRPPEESTQEMMEEEEEIPKPKS AVAPPGAPKKEHVNVVF 216
	Gspt2 part corresponding to Sup35NM	1 MDLGSSSDSAPDCWDQVDMEAPGSAPSGDGIAPAAMAAAEAAEAEAQRKHLSLAFSSQLNIHAKPFVP SVSAAEFVPSFLPGSAQPPAPTASSCDETCIGGAGEPEGKRMEWGAPVEPSKDGPLVSWEGSSSVVTM ELSEPVVENGEVEMALEESWELKEVSEAKPEASLGDAGPPEESVKEVMEEKEEVRKSKSVAIPSGAPK KEHVNVVF 212

* **Related to Figure 5**.

Placental mammals have two Sup35 homologs, Gspt1 and Gspt2. Gspt1 is expressed in most tissues in a proliferation-dependent manner. Gspt2, a result of retro-transposition of a *GSPT1* mRNA, is expressed almost exclusively in the brain and does not respond to growth stimulation. Paradoxically, while Gspt1 appears to be a major translation termination factor, only Gspt2 can restore the viability of *sup35-Δ* yeast strains (Hoshino et al., 1998; Le Goff et al., 2002; Zhouravleva et al., 2006).

** **For yeast *S. cerevisiae* Sup35NM.**

Sup35N is divided into three parts: (i) the Q/N-rich N-terminal part critical for aggregation and the formation of the [*PSI*$^+$] prion (aa 1-40), (ii) oligopeptide repeats that promote the *de novo* appearance of [*PSI*$^+$] and are also implicated in its maintenance (aa 41-97; repeated oligopeptides underlined), and (iii) the part encompassing the N/Y-rich and Q/G-rich stretches overlapping with the secondary aggregation region and region required for the faithful propagation of the [*PSI*$^+$] prion variants (DePace et al., 1998; Li and Lindquist, 2000; Parham et al., 2001; Krishnan and Lindquist, 2005; Shkundina et al., 2006; Tessier and Lindquist, 2007; Chang et al., 2008).
Sup35M is also divided into three parts: (i) the K-rich part (aa 124-166) encompassing the Q/K and K-rich stretches (underlined); recent evidence suggest that this region directly interacts with the Hsp104 chaperone (Helsen and Glover, 2012); (ii) the E/K rich part (aa 167-222, conserved mixed K/E patches are underlined); and (iii) the region adjacent to Sup35C (aa 223 – 253; underlined are the N-rich patch and the E-rich sequence). See Results for a more detailed discussion of regions within Sup35M. See Fig. 5B for graphic presentation of structural features in Sup35NM.

*** **For parts of mouse Gspt1 and Gspt2 corresponding to Sup35NM.**

Underlined are sequences that contain both E and K residues in Gspt2, and corresponding sequences in Gspt1 that are mostly E-rich.
Highlighted in yellow is the fragment of Gspt2 used in the Sup35N-Gspt2M-Cfp fusion construct in Fig. 5B.
Numbers indicate amino acid residues. The Q, N, K and E residues are shown in green, brown, red and blue, respectively.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains

Unless otherwise stated strains are isogenic derivatives of 74-D694 (***a*** *ade1-14* (UGA) *his3-Δ200 ura3-52 leu2-3, 112 trp1-289* (UAG); Chernoff et al., 1995), and the [*pin*$^-$][*psi*$^-$] derivative was used unless the presence of [*PIN*$^+$] or [*PSI*$^+$] is indicated. The [*pin*$^-$][*psi*$^-$] 74-D694 (1G4) derivative was obtained from the original [*PIN*$^+$][*psi*$^-$] 74-D694 (1Y1) isolate by growth on 5 mM GuHCl (Derkatch et al., 1997). Growth on media with 5 mM GuHCl eliminates [*PIN*$^+$], as well as other prions (Tuite et al., 1981; Derkatch et al., 1997). [*PSI*$^+$][*pin*$^-$] derivatives (L1759 and L1763 for weak and strong [*PSI*$^+$], respectively) were obtained from [*PIN*$^+$][*psi*$^-$] (1Y1) by the overexpression of the Sup35 prion domain and selection for [*PSI*$^+$], followed by 5mM GuHCl treatment and selection of clones that lost [*PIN*$^+$] but retained [*PSI*$^+$] (Derkatch et al., 2000).

The *hsp104-Δ* (YID188) strain was obtained by substituting the *HIS3* marker for the entire *HSP104* ORF in 74-D694 (Potenski and Derkatch; in preparation); the strain is [*pin*$^-$][*psi*$^-$] due to lack of Hsp104. The [*pin*$^-$][*psi*$^-$] strain expressing Yfp-tagged Dcp2 in chromosomal location was obtained from the [*PIN*$^+$][*psi*$^-$] *DCP2-YFP* (YID189) strain by growth on 5mM GuHCl to eliminate [*PIN*$^+$]. YID189 was made by integrating the pDCP2-YFP *URA3*-marked pRS406-based integrative plasmid into the chromosomal *DCP2* gene in [*PIN*$^+$][*psi*$^-$] 74-D694. As a result of integration, the *DCP2-YFP* construct is under the control of the *DCP2* promoter, and the endogenous gene is not expressed due to lack of promoter (Potenski and Derkatch, in preparation). The *hsp104-Δ DCP2-YFP* (YID190) strain was made the same way as YID189 but starting with the *hsp104-Δ* (YID188) strain (Potenski and Derkatch, in preparation).

The [*pin*$^-$] *sup35-ΔNM* strain was obtained from [*PIN*$^+$] 74-D694 *sup35-ΔNM* by eliminating [*PIN*$^+$] by growth on 5 mM GuHCl. The [*PIN*$^+$] *sup35-ΔNM* strain (Derkatch et al., 1997) was obtained from [*PIN*$^+$][*psi*$^-$] (1Y1) derivative by the integration-excision method.

The [*pin*$^-$][*psi*$^-$] *sup35-Δ* strain was obtained from the YID147 [*PIN*$^+$][*psi*$^-$] *sup35-Δ* strain by growth on 5 mM GuHCl, to eliminate [*PIN*$^+$]. The YID147 strain was constructed from [*PIN*$^+$][*psi*$^-$] 74-D694 by the integration-excision method using the integrative plasmid with sequences immediately upstream and downstream of the *SUP35* ORF (Zadorski and Derkatch, unpublished). The viability of the strain is maintained by the pYCH-U2 *URA3* centromeric plasmid expressing the *SUP35* ORF driven by the original *SUP35* promoter (Chernoff et al., 1995). Centromeric *LEU2* vectors expressing *SUP35* or the *SUP35-ΔN* fragment controlled by the original *SUP35* promoter (Zadorski and Derkatch, unpublished) were substituted for the full-length *SUP35* by plasmid shuffle using the FOA media to select for the loss of the *URA3* vector (Boeke et al., 1984).

The *pub1-Δ* strain is a single-step disruption obtained in [*pin*$^-$][*psi*$^-$] 74-D694 by substituting the *HIS3* marker for the entire *PUB1* ORF. The disruption fragment flanked by sequences immediately upstream and downstream of the *PUB1* ORF was obtained by amplifying *HIS3* from pRS413 with primers #377 and #378 (see Supplementary Primers Table below for primer sequences). Disruption was confirmed by PCR amplification of the chromosomal DNA fragments in the *PUB1* region with primers #379, 387, 380, 388.

Spontaneous *sup35* and *sup45* mutants were selected by their ability to suppress both UGA (*ade1-14*) and UAG (*trp1-*289) nonsense mutations essentially as described previously (Inge-Vechtomov, 1988). Individual colonies of [*pin*$^-$][*psi*$^-$] 74-D694 were patched on YPD and replica plated to SD-Ade,Trp. The Ade$^+$Trp$^+$ colonies were picked after 7-14 days of incubation at 30^{0}C. To determine the presence and location of *sup35* and *sup45* mutations, *SUP35* and *SUP45* genes were amplified by PCR (primers # 385 and 386, and #414 and 415, respectively); PCR products were sequenced at Genewiz and sequences were analyzed with the ApE software.

The strain expressing chromosome-encoded *PUB1-GFP* in BY4741 background was purchased from Invitrogen (Cat. 95700YNL016W, Huh et al., 2003); *PUB1-GFP* is controlled by the endogenous *PUB1* promoter.

Plasmids

Unless specifically mentioned, centromeric vectors from the pRS400 series were used as backbones for constructs made for this study (Sikorski and Hieter, 1989), and the ORFs are controlled by the *CUP1* promoter. In all plasmids the modules encompassing the *CUP1* and *GAL1* promoters, and the *YFP*, *GFP* and *CFP* ORFs are the same as in the constructs described previously (Derkatch et al., 2004; Kadnar et al., 2010). *CUP1* and *GAL1* are *Eco*RI - *Bam*HI inserts, and fluorescent protein ORFs are cloned between the *Sac*II and *Sac*I sites. Analogously, the *SUP35* promoter in the pSUP35-SUP35 and pSUP35-SUP35-ΔN is also cloned as the *Eco*RI - *Bam*HI insert. And the ORF for the mCherry fluorophore (amplified from pmCherry (Clontech) with primers #340 and 341) is inserted into pCUP-TIA1-mCHERRY as a *Sac*II-*Sac*I fragment. Only in the control pCUP-YFP and pCUP-CFP plasmids flourophore ORFs are inserted as *Bam*HI-*Sac*I fragments, so that they immediately follow the promoter.

The *TIA1*, *PUB1*, *SUP35* and *RNQ1* ORFs and ORF fragments are cloned as *Bam*HI - *Sac*II inserts; in the untagged constructs stop codons precede the *Sac*II site. Unless specifically mentioned, plasmids bearing *PUB1, TIA1* and their fragments are *URA3*-marked, and plasmids bearing *SUP35* and its fragments, *GSPT*s and the *URE2(1-65)-SUP35M* fusion are *LEU2*-marked.

See below Supplementary Plasmids Table for more information on plasmid construction and Supplementary Primers Table for primer sequences.

To construct Tia1 expression vectors, total C57BL6/J mouse brain RNA was extracted with TRIzol® Reagent (Invitrogen, 15596-018), and cDNA was prepared with the SuperScript® III One-Step RT-PCR reagents (Invitrogen, 12574) according to manufacturers' protocols. *mTIA1* (NM_001164079) was cloned as PCR product.

To construct pCUP-PUB1-YFP (*URA3*), yeast chromosomal DNA was purified as described in Looke et al. (Looke et al., 2011), and *PUB1* ORF was amplified by PCR.

Mouse *GSPT1* (BC031640.1) and *GSPT2* (BC117825.1) were PCR amplified from clones purchased from Open Biosystems.

A set of *CEN URA3* plasmids encompassing the first exon of huntingtin with polyQ stretches of different length (25, 47, 72 and 103 aa), tagged with *GFP* and driven by the constitutive *GPD* promoter were kindly provided by S. Lindquist, MIT (Krobitsch and Lindquist, 2000). These *URA3*-marked plasmids were co-expressed with a *LEU2*-marked version of pCUP-TIA1-YFP.

Yeast cultivation, genetic methods and analysis of aggregate formation

Standard yeast media and cultivation procedures were used (Amberg et al., 2005). Unless specifically mentioned, yeast were grown at 30^0C. Also, unless specifically mentioned, untransformed strains, as well as all cultures for protein and RNA isolations were grown in complete organic medium, YPD, and cultures of transformants for the analysis of protein aggregation were grown in synthetic glucose media selective for plasmid maintenance. The *CUP1* promoter was induced by adding $CuSO_4$ into the plasmid-selective media to a final concentration of 50 μM in liquid media and 20 μM in solid media (these concentrations give similar level of protein expression under these growth conditions). The *GAL1* promoter was

induced with 2% galactose in media lacking glucose but supplemented with 1% sucrose and 1% raffinose (SGalSR; all experiments were in liquid media).

To analyze formation of Tia1, Pub1, Sup35, Gspt1 and Gspt2 aggregates, transformants carrying respective expression constructs were pre-cultured in liquid plasmid-selective media to early stationary phase (~ 2 days), then cultures were diluted to OD_{600} ~ 0.1, and aggregation was monitored until cultures reached stationary phase (~2 days). See Figure Legends for details of specific experiments. When analyzing aggregate formation, for all strains and constructs representative groups of cells were scored, including cells of different size, with and without buds and with different levels of construct expression. Unless specifically mentioned, differences in aggregation patterns and frequency were not due to an obvious prevalence of a particular subset of cells in terms of size, budding or protein expression levels. Also, unless specifically mentioned, for imaged cells, differences in size, budding and fluorescence intensity are within population variability range.

Sensitivity of the *sup35* and *sup45* mutants and *pub1-Δ* and *sup35-ΔNM* strains to benomyl was analyzed on solid YPD media supplemented with 30, 40 or 50 mg/l of benomyl (Fluka, 45339); growth was analyzed several times between days 1 and 5 of incubation at 30^0C. Effects of benomyl on the formation of Tia1 / Sup35 aggregates were analyzed in liquid cultures supplemented with 20 mg/l benomyl; see Figure 6B and Supplementary Figure S6A legends for details.

Sensitivity to latranculin A was analyzed as described in Bailleul-Winslett et al., 2000; see Supplementary Figure S6B legend for details.

Nonsense suppression due to *sup35* and *sup45* mutations was analyzed on SD-Trp and SD-Ade after 2-5 days of growth at 30^0C. Nonsense suppression due to the presence of [*PSI*$^+$] was analyzed on SD-Ade and on SEt-Ade (2% ethanol substituted for dextrose) after 5 – 21 days of growth at 30^0C or 20^0C.

For [*PSI*$^+$] induction assays, [*PIN*$^+$][*psi*$^-$] and [*pin*$^-$][*psi*$^-$] derivatives were transformed with a plasmid encoding a protein to be tested, pCUP-TIA-YFP (or the pCUP-YFP control), and a plasmid encompassing a [*PSI*$^+$]-inducing construct, pCUP-SUP35NM-CFP (or the pCUP-CFP control). Transformants were grown for 3 days on solid plasmid-selective media supplemented with 20 μM $CuSO_4$ and then transferred to adenineless media (SD-Ade and SEt-Ade) where growth was possible due to the suppression of the *ade1-14* nonsense mutation, and thus was indicative of the *de novo* appearance of [*PSI*$^+$]. Suppression was scored multiple times during ~20 days of growth at 30^0C or 20^0C. Curing of the Ade$^+$ phenotype by GuHCl was used to confirm that suppression was due to [*PSI*$^+$].

To analyze [*PSI*$^+$] maintenance, transformants of [*PSI*$^+$] derivatives bearing pCUP-TIA-YFP were grown for at least 25-30 generations in media supplemented with 50μM $CuSO_4$, and then were colony purified on YPD. On this medium, [*psi*$^-$] *ade1-14* yeast form red colonies, whereas suppression of the mutation by [*PSI*$^+$] results in white or pink colonies. Appearance in [*PSI*$^+$] cultures of red colonies, or of white / pink colonies with red sectors would be indicative of [*PSI*$^+$] destabilization.

Pseudohyphal growth was analyzed by microscopy after growth on solid YPD medium for 2 days.

To stain bud scars, yeast were grown in liquid SD media at 30^0C until late log phase (for ~30 hrs). Calcofluor A (Sigma F3397) was added to a final concentration of 100 μg/ml, cells were incubated with the dye for 10 min at room temperature and then washed with water 3 times and observed with the DAPI filter set (Amberg et al., 2005).

Isolation of total yeast DNA for PCR amplifications

DNA was prepared according to Looke et al., 2011: a 15 min extraction in 200 mM LiOAc / 1% SDS at 70^0C followed by ethanol precipitation.

Preparation of yeast cell lysates and electrophoresis

Yeast were harvested at the indicated time points or stages of culture growth. Cells were washed in ice-cold water and re-suspended in the protein extraction buffer (50 mM Tris-HCl, pH 7.5; 50 mM KCl and 10 mM $MgCl_2$) supplemented with 10mM phenylmethylsulfonyl fluoride and Complete™ protease inhibitor cocktail (Roche Applied Science), to prevent protein degradation. Lysates were prepared by vortexing cells with glass beads as described previously (Liebman et al., 2006) and pre-cleared by centrifugation at 10,000 x g for 5 min at 4^0C. When samples were intended for subsequent RNA amplification, all reagents were treated with DEPC.

SDS-AGE gel electrophoresis of whole cell lysates was performed as previously described (Liebman et al., 2006). BioRad transfer chamber was used for wet electrophoretic protein transfer. Prior to loading on the gel, lysates (~40 μg of total protein) were incubated in the sample buffer (25 mM Tris; 200 mM glycine, pH 8.3; 2% SDS, 5% glycerol and 0.025% bromphenol blue) at room temperature (~25^0C) for 7 min. To dismantle aggregates held together by hydrophobic bonds, control samples were incubated in boiling water bath instead of the room temperature incubation. As suggested by published protocols (Bagriantsev et al., 2004), major components of chicken pectoralis extract were used as molecular weight markers on SDS-AGE gels (a kind gift form Dr. Keller; Kim and Keller, 2002). Ponceau-S staining of the membranes detects the prevailing proteins in the extract: the titin dimer at ~3000 kDa (usually not well seen), the nebulin at ~750 kDa, and the myosin heavy chain at ~200 kDa. The markers are distributed on SDS-AGE gels as smears, allowing for only an approximate estimate of oligomer size.

SDS-PAGE gel electrophoresis and Western blotting was carried out using Mini-protein 3 system (BioRad) according to manufacturer's protocols.

Odyssey (LI-COR Inc) system was used for imaging and image analysis.

Analysis of protein sedimentation on sucrose gradients

Analysis of protein sedimentation on sucrose gradients was performed according to standard protocols (Hwang and Murray, 1997). Approximately 800 μg of total protein lysates were resuspended in 500 μl of protein extraction buffer and loaded onto 12.5 ml 5% to 40% step sucrose gradient prepared in the same buffer (50 mM Tris-HCl, pH 7.5; 50 mM KCl and 10 mM $MgCl_2$) and supplemented with 10mM phenylmethylsulfonyl fluoride and Complete™ protease inhibitor cocktail (Roche Applied Science). After centrifugation at 237,000 x g for 4 hours at 4^0C, 1 ml fractions were collected, proteins were precipitated with trichloroacetic acid and then resuspended in the SDS-PAGE sample buffer for subsequent Western analyses.

Immunoprecipitation and analysis of Tub1-associated complex

Yeast cell lysates (~ 200 μg of total protein) were incubated with primary antibody in 1 ml of immunoprecipitation buffer (IP; 50 mM Tris-HCl, pH 8; 150 mM NaCl; 0.1mM DTT; 0.1% Triton-X 100) for 1 hr at 4^0C. Then pre-cleaned magnetic beads (Dynabeads Protein-G, Invitrogen 100-03D) were added, and the slurry was rotated for 1 hr at 4^0C. Dynabead-associated immunoprecipitates were transferred to new tubes and washed 6 times with the IP buffer. When RNAse treatment is mentioned, samples were incubated for 1 hr at room temperature with 0.1 mg/ml RNAse A prior to immunoprecipitation.

For subsequent SDS-PAGE, proteins were eluted into the 2xSDS-PAGE sample buffer (boiling water bath, 5 min). For subsequent SDS-AGE, proteins were transferred into the 2%

SDS SDS-AGE buffer and beads were removed by a magnet (in this buffer elution is very quick and efficient at room temperature).

RNA was eluted from immunoprecipitates with TRIzol® Reagent (Invitrogen), and cDNA was prepared with SuperScript® III OnepStep RT-PCR reagents (Invitrogen, Cat.12574), according to manufacturers' protocols. Specific mRNAs were amplified by PCR; two different sets of primers were used for each mRNA.

Antibodies

The following primary antibodies were used for immunodetection and immunoprcipitation: anti-Tia1 (1:500, Santa Cruz sc-1751, raised in goat, polyclonal), anti-RPL28 (1:1000, a gift from J. Warner, Albert Einstein College of Medicine, New York, NY; raised in rabbit), anti-Sup35 (BE4, 1:1000, a gift from S. Liebman, University of Nevada, Reno; yeast, raised in mouse, monoclonal, recognizes Sup35C; Bagriantsev and Liebman, 2006), anti-Sup35N (a gift from S. Lindquist, MIT, yeast, anti-peptide, raised in rabbit; Patino et al., 1996), anti-actin (1:1000, Sigma A5060; raised in rabbit, polyclonal), anti-α-Tubulin (YOL 1/34; 1:1000, Thermo MA1-80189; yeast, raised in rat, monoclonal), anti-Gfp (1:1000, Clontech 632375; raised in mouse, monoclonal), anti-mCherry (1:1000, Clontech 632496, raised in rabbit, polyclonal), anti-Rnq1 (1:25000, Type 2, a gift from S. Lindquist, MIT, yeast, raised in rabbit, polyclonal); anti-eIF4e (1:1000, Abcom ab1126, raised in rabbit, polyclonal); anti-Pab1 (1:1000, EnCor Biotechnology, yeast, raised in mouse, monoclonal);. All secondary antibodies were from LI-COR with IRDye 680 or 800.

Light and fluorescence microscopy

Cells were observed using a confocal laser scanning microscope FV1000 (Olympus) with a 60x / 1.42 NA oil immersion objective. Images were analyzed with FV10-ASW software. Differential interference contrast (DIC) images are shown for light microscopy.

Recombinant Tia1 purification, in vitro fiber formation and transmission electron microscopy

To express Tia1 in *Escherichia coli*, mouse Tia1 ORF was inserted into the pET-SUMO vector (Invitrogen). BL21-AI One Shot cells (Invitrogen) carrying the pET-SUMO-TIA1 construct were grown in LB medium supplemented with 100 μg/ml ampicillin at 37°C to OD_{600} ~ 0.6, then expression of the *6xHIS-SUMO-TIA1* fusion was induced by 1mM IPTG and 0.2% L-arabinose for 2 hours. Cells were harvested and lysed by gentle agitation in the lysis buffer (8ULB; 100mM NaH_2PO_4 pH 7.4, 200mM NaCl, 8M urea) for 1hour. After removal of cell debris, supernatant was incubated for 2 h with Ni-NTA Sepharose (Qiagen) equilibrated with 8ULB. The slurry was transferred to the column, washed extensively with 8ULB, and 6xHis-Sumo-Tia1 was eluted with 8ULB containing 250mM imidazole. Protein enriched fractions were determined by UV absorption at 280nm. After gradual dialysis at 4^0C with Slide-A-Lyzer (20K MWCO, Thermo, 87734) into PBS pH 7.4 with 1M urea, the 6xHis-Sumo part was cut-off with the His-tagged Sumo protease 1 (LifeSensors; incubation at 30°C for 2 hrs). To remove 6xHis-Sumo and the protease, the mixture was incubated with Ni-NTA Sepharose equilibrated with PBS pH7.4, 1M urea.

After gradual dialysis of Tia1 into PBS pH 7.4, 0.2 M urea, 100 μl reactions (~20μg of protein) were set up in the assembly buffer (PBS pH7.4 with 0.1M urea) at 4^0C for 2hr.

TEM was performed at the NYU Langone Medical Image Core Facility. Fiber suspensions were diluted 2.5-fold in water, and 4 μl were applied onto the carbon-coated 400 mesh Cu/Rh grids (Ted Pella Inc.). The grids were washed 3 times with water to get rid of urea,

and negatively stained with 1% uranyl acetate (twice briefly, then 5 min at 25°C). Images were obtained with Philips CM12 transmission electron microscope supplied with Gatan 1k×1k digital camera and processed using Gatan Digital Micrograph software.

Primers used in this study. Supplemental Experimental Procedures Table*.

Primer #	Sequence
320	5’ GGATCCATGAGTAAAGGAGAAGAA
321	5’ GAGCTCTCATTTGTATAGTTCATCC
324	5’ CCGCGGTCACTCGGCAATTTTAACAATTTTA
329	5’ GTGCCGCGGCTGGGTTTCATACC
340	5’ CCCGGATCCATGGTGAGCAAGGGCGAG
341	5’ CCCGAGCTCTTACTTGTACAGCTCGTCCATGC
342	5’ CCCGGATCCATGTCTGAAAATAACGAAGAACAACA
343	5’ GTGCCGCGGTTGTTGTTGCTGCTGTTGC
347	5’ CAATCAATCAGGATCCACAATGTCGGATTCA
348	5’ GGGAACAAAAGCTGGAGCTCTCACATATCGTTAACAACTTCGTCATCCA
357	5’ CAATCAATCAGGATCCACAATGTCTTTGAACGACTTTCAAA
358	5’ CTTCTCCTTTACTCCGCGGCATATCGTTAACAACTTCGTCA
359	5’ GATTGGTGGTGGATCCATGGAGGACGAGATGCC
360	5’ AATACCTAAGCTTCCGCGGTCACTGGGTTTCATACCCG
361	5’ CTTCTCCTTTACTCCGCGGACCTTGAGACTGTGGTTGGA
364	5’ CAATCAATCAGGATCCATGATAAACCCTGTGCA
365	5’ CTTCTCCTTTACTCCGCGGCTGGGTTTCATA
366	5’ CAATCAATCAGGATCCATGGAGGACGAGATGCCCA
367	5’ CTTCTCCTTTACTCCGCGGATCAAGAGTCTCTT
370	5’ CAATCAATCAGGATCCATGGCTGCGGTGGCCGAGGCCCA
371	5’ CTTCTCCTTTACTCCGCGGGTCTTTCTCTGGAACCA
372	5’ CAATCAATCAGGATCATGGATCTCGGCAGCAGCAGCGA

373	5’ CTTCTCCTTTACTCCGCGGGTCCTTCTCTGGGACCAGCT
374	5’ ACAGTCTCAAGGTCCAATGGAACTTTCAGAACCT
375	5’ CTTCTCCTTTACTCCCGAAGACCACATTTACGTGTTCT
377	5’GAAGATTACCACATCTACTCTTTGTTCGATTCCACTCTTGGCCTCCTCTAG
378	5’ CTTTCTTTTTGTTTCATTCCACTTTTCTTCATAATATTCGTTCAGAATGACACG
379	5’ GAAGATTACCACATCTA
380	5’ CATTCCACTTTTCTTCAT
387	5’ CGTGCTTTAAGTTAGAGTTTAATCTTCCCT
388	5’ ATCTTGGGATTTGTAGGTTGCCTCT
391	5’ CACGTAGATCCAAAAGAATGCCCA (Sup35 RT-PCR)
392	5’ CAACTTCATTTTCAGTTTCGT (Sup35 RT-PCR)
394	5’ CAATCAATCAGGATCCATGTTTGGTGGTAAAGA
395	5’ CTTCTCCTTTACTCCGCGGCTCGGCAATTT
398	5’ AGGGCCGTCTGTATGTTGTC (Tub1 RT-PCR)
399	5’ GGCACCCACTTCGATGTAAT (Tub1 RT-PCR)
400	5’ AAGCTTTCATCAAATGCAGGTT (Tub1 RT-PCR)
401	5’ AAGTGGGTGCCGACTCATAC (Tub1 RT-PCR)
406	5’ ACAGTCTCAAGGTCCCGAATCTGATAAGAAAGAGGA
407	5’ CTTCTCCTTTACTCCGCGGGATTTTAAGGTCTTCTACCT
410	5’ CAATCAATCAGGATCCATGGAATCTGATAAGAAAGAGGA
412	5’ GCCGAATCTGATAAGAAAGAGGA (Sup35 RT-PCR)
413	5’ CATTGGCATTGTTGGTATTATGTG (Sup35 RT-PCR)
444	5’ CAGGATCCATGTCTGAAAATAACGAAG
445	5’ CCGCGGATCACGCTTAGCAGC

446	5’ CCGCGGTGGCATAAAATTTGATCT
456	5’ CAATCAATCAGGATCATGATGAATAACAACG
457	5’ AAGACATTGTGGATCCCGGCCGCTGTTATTGTTTTG
463	5’ GAGTCGAGTTGTTTGGGAATGC (rRNA RT-PCR)
464	5’ TCTCTTTCCAAAGTTCTTTTCATCTTT (rRNA RT-PCR)
465	5’ GCATATCAATAAGCGGAGGAAAAG (rRNA RT-PCR)
466	5’ GGTCCGTGTTTCAAGACGG (rRNA RT-PCR)
467	5’ TCCGTAGGTGAACCTGCGG (rRNA RT-PCR)
468	5’ TCCTCCGCTTATTGATATGC (rRNA RT-PCR)
473	5’ GCCAGCTATCAGAAGATTAGCT (*HHF2* RT-PCR)
474	5’ CCACCGAAACCATATAAGGTTCT (*HHF2* RT-PCR)
475	5’ CTGCTGTCTTGGAATATTTGGC (*HTA1* RT-PCR)
476	5’ CTTGAGAAGCCTTGGTAGCC (*HTA1* RT-PCR)
481	5’ GTTGGAAAAGAACGACAGAAGATCTGGT (*TEF1* RT-PCR)
482	5’ CAGTCTTGTCAACAGACTTGATAACACCG (*TEF1* RT-PCR)
483	5’ GTGGACATTGATCCCAGAAGACAAGA (*RPL28* RT-PCR)
484	5’ CTGATTTTTTCTTCAGCCAACTTGGAGA (*RPL28* RT-PCR)
485	5’GAACCAGTTCCAGTCCAGACTTCGGAACTTCCAAAGGTAGAAGACCTTAAAA TCCCGCGGAGTAAAGGAGAAG
486	5’ACAGTCTCAaGGTCCCGAATCTGATTCTGCTGAAACCCCAACTCCAACAAAG GTCGAAGAACCAGTTCCAGTCCAGA
501	5’ GCCAGCTATCAGAAGATTAGCT
502	5’ CCACCGAAACCATATAAGGTTCT
503	5’ CTGCTGTCTTGGAATATTTGGC

504	5’ CTTGAGAAGCCTTGGTAGCC

* Primers used for RT-PCR are indicated; all other primers are mentioned either in the Strains and Plasmids sections of the Supplemental Experimental Procedures, or in the Plasmid Construction Table below.

Plasmid construction. Supplemental Experimental Procedures Table

Plasmid	Yeast marker	Inserted ORF or ORF fragment	Primers, plasmid used to clone the ORF, or reference
pCUP-YFP	*URA3*	*YFP*	320; 321; see text*
pCUP-CFP	*LEU2*	*CFP*	320; 321; see text
pCUP-TIA	*URA3*	*TIA1*	359; 360; see text
pCUP-TIA-YFP	*URA3*	*TIA1*	359; 329**
pCUP-TIA-YFP (LEU2)	*LEU2*	*TIA1*	359; 329**
pCUP-TIA-mCHERRY	*URA3*	*mCherry*	340, 431; see text
pGAL-TIA1-YFP	*URA3*	*GAL* promoter	See text
pCUP-TIA1RBD-YFP	*URA3*	*TIA1* (1-289)	366; 367**
pCUP-TIA1PRD-YFP	*URA3*	*TIA1* (290-386)	364; 365**
pCUP-PUB1-YFP	*URA3*	*PUB1*	342; 343**; see text
pCUP-SUP35NM-CFP	*LEU2*	*SUP35* (1-253)	Derkatch et al., 2004
pGAL-SUP35NM-CFP	*LEU2*	*SUP35* (1-253)	Kadnar et al., 2010
pCUP-SUP35-CFP	*LEU2*	*SUP35*	347; 395**
pCUP-SUP35	*LEU2*	*SUP35*	347; 324; see text
pCUP-SUP35N-CFP	*LEU2*	*SUP35* (1-123)	347; 361**
pCUP-SUP35M-CFP	*LEU2*	*SUP35* (124-253)	357; 358**
pCUP-SUP35C-CFP	*LEU2*	*SUP35* (254-685)	394; 395**
pCUP-SUP35(1-153)-CFP	*LEU2*	*SUP35* (1-153)	pID65 by ILD**
pCUP-SUP35(166-220)-CFP	*LEU2*	*SUP35* (1,166-220)	410; 407**
pCUP-SUP35(N+166-220)-	*LEU2*	*SUP35* (166-	406; 407; into pCUP-

CFP		220)***	SUP35N-CFP
pCUP-SUP35(N+166-254)-CFP	*LEU2*	*SUP35* (166-254)***	406; 348; into pCUP-SUP35N-CFP
pCUP-SUP35NM(N+166-220-ΔK/E)	*LEU2*	*SUP35* (166-254-ΔK/E)***	485; 486 into pCUP-SUP35N-CFP****
pCUP-RNQ1-CFP	*HIS3*	*RNQ1*	Kadnar et al., 2010
pCUP-mGSPT1-CFP	*LEU2*	*mGSPT1*	370; 371**; see text
pCUP-mGSPT2-CFP	*LEU2*	*mGSPT2*	372; 373**; see text
pCUP-SUP35N-mGSPT2(136-212)-CFP	*LEU2*	*mGSPT2*(136-212)***	374, 375; into pCUP-SUP35N-CFP
pSUP35-SUP35	*LEU2*	*SUP35*	See text
pSUP35-SUP35-ΔN	*LEU2*	*SUP35-ΔN*	See text
pET-SUMO-TIA1	None	*TIA1*	359; 360; see text
pCUP-URE2(1-65)-SUP35M-CFP	*LEU2*	*URE2* (1-65)***	456, 457; into pCUP-SUP35M-CFP; see text
pGPD-Q25-SUP35M-GFP	*URA3*	Ht exone 1; Q25	Krobitsch and Lindquist, 2000
pGPD-Q47-SUP35M-GFP	*URA3*	Ht exone 1; Q47	Krobitsch and Lindquist, 2000
pGPD-Q72-SUP35M-GFP	*URA3*	Ht exone 1; Q72	Krobitsch and Lindquist, 2000
pGPD-Q103-SUP35M-GFP	*URA3*	Ht exone 1; Q103	Krobitsch and Lindquist, 2000
pCUP-PUB1(1-419)-YFP	*URA3*	*PUB1(1-419)*	444, 446**

pCUP-PUB1(1-245)-YFP	*URA3*	*PUB1(1-245)*	444, 445**

* Construction of these plasmids is described above in the Plasmids section of the Supplemental Experimental Procedures.

** All these constructs were made the same way: an ORF or ORF fragment was amplified with the indicated primers, or excised from the indicated vector, and inserted into the *Bam*HI and *Sac*II sites of the vector already carrying the indicated promoter (as an *Eco*RI – *Bam*HI fragment) and flourophore ORF (as a *Sac*II – *Sac*I fragment). Vectrors used to clone the insertions were described previously (Derkatch et al., 2004; Kadnar et al., 2010).

*** For these plasmids the ORF fragment inserted at the last step of plasmid construction is only part of the *SUP35* ORF fragment in the resulting plasmid.

**** Primers were annealed to each other and the whole insert was obtained by an extension reaction.

Supplemental References
(only references not in the main text are included)

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